REF IS-4000	IDS-iSYS Intact PINP	
Instructions for Use	*IN VITRO DIAGNOSTIC*	CE

Intended Use

For In Vitro Diagnostic Use

The IDS-iSYS Intact amino-terminal propeptide of type I procollagen (Intact PINP) assay is intended for the quantitative determination of intact PINP in human serum or plasma on the IDS-iSYS Multi-Discipline Automated Analyser.

Summary and Explanation

An important step in the bone formation process is synthesis of type I collagen, which is the major organic component in bone matrix. During collagen synthesis, propeptides are released from both the amino- and the carboxyterminal parts of the procollagen molecule [1]. These propeptides are secreted into the blood circulation. The amino-terminal propeptide of type I procollagen (PINP) is probably the most specific and sensitive marker of bone formation [2]. PINP is a particularly useful marker for monitoring the efficacy of osteoporosis therapy with anabolic agents [3,4], but it is also one of the best bone turnover markers for monitoring the efficacy of antiresorptive therapy [4,5].

Method Description

This assay is based on chemiluminescence technology. Samples and calibrators are diluted in a diluent and a portion of this is incubated with a biotinylated anti-PINP monoclonal antibody, an acridinium labelled monoclonal antibody, streptavidin labelled magnetic particles and an assay buffer. The magnetic particles are captured using a magnet and a wash step performed. Trigger reagents are added and the resulting light emitted by the acridinium label is directly proportional to the concentration of intact PINP in the original sample.

Warnings and Precautions

The IDS-iSYS Intact PINP is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in these Instructions for Use (IFU). IDS Ltd. will not be held responsible for any loss or damage (except as required by statute), howsoever caused arising out of non-compliance with the instructions provided.

CAUTION: This kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practice must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Sodium Azide

Xn. Harmful: Calibrators CAL contain sodium azide (NaN_3) >0.1 % (w/w) (<1 %).

R22	Harmful if swallowed.
R52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S46	If swallowed, seek medical advice immediately and show this container or label.
S36/37	Wear suitable protective clothing and gloves.
S60	This material and/or its container must be disposed of as hazardous waste.

Some reagents in this kit contain sodium azide as a preservative, which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Handling Precautions

Apart from the calibrators, which are lyophilised, the reagents provided in the kit are ready to use. Refer to the Calibrator section of the procedure for reconstitution methodology.
Before a new cartridge is loaded onto the Analyser, the magnetic particle container requires mixing by the operator with a brisk rotation motion. This will resuspend the magnetic particles that have settled during shipment. It is very important to avoid any foam formation.

Shelf Life and Storage of Reagents

Prior to first use, store the cartridge and the calibrators in an **upright** position in the dark at 2 - 8 °C. Do not freeze the cartridge or the calibrators.

Reagent shelf life	Cartridge	Calibrators
Before opening at 2 - 8 °C	To the expiry date	
Cartridge, after opening at 2 - 8 °C	28 Days	N/A
Calibrators, after reconstitution at 2 - 8 °C	N/A	4 weeks
On board the Analyser **(*)**	7 Days	3 hours

(*) Continuous on board stability.

Sample Collection and Storage

The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin, ammonium heparin or potassium EDTA) samples. Samples should be separated as soon as possible after collection.
Store samples at -20 °C. Avoid repeated freeze - thaw of samples.
Samples containing particulate matter must be centrifuged before performing the assay.
Do not use heat-inactivated samples.
To minimise possible evaporation effects, samples, calibrators, and controls should be measured within 3 hours of being placed on the Analyser.
Before performing assays, make sure that samples, calibrators and controls are at room temperature (20 - 25 °C).
Note: Some sample collection tubes that are commercially available might affect the results of testing in particular cases.
It is recommended to follow the instructions of the tube manufacturer especially when processing samples in primary tubes.

Procedure

Materials Provided

Reagent Cartridge

MPS3

Magnetic particles coated with streptavidin in phosphate BSA buffer with sodium azide as preservative (<0.1 %), 1 bottle, 2.3 mL.

CONJ

Anti-PINP monoclonal antibody labelled with an acridinium ester derivative, in buffer containing bovine, and mouse proteins with sodium azide as preservative (<0.1 %), 1 bottle, 4.95 mL.

Ab-BIO
Anti-PINP monoclonal antibody labelled with biotin, in buffer containing mouse and bovine proteins with sodium azide as preservative (<0.1 %), 1 bottle, 16.8 mL.

BUF
Buffer containing mouse and bovine proteins, with sodium azide as preservative (<0.1 %), 1 bottle, 4.95 mL.

DIL
Buffer containing sodium azide as preservative (<0.1 %), 1 bottle, 23.7 mL.

Calibrators
CAL A
CAL B
Lyophilised buffer containing BSA with <1.0 % sodium azide as preservative (<0.05 % reconstituted). 2 each of 2 concentration levels, 1.0 mL per bottle.

Mini CD
Contains IFU for IDS-iSYS reagents, control ranges and XML files.

Materials Required But Not Provided

IDS-iSYS Multi-Discipline Automated Analyser: IS-310400
IDS-iSYS PINP Control Set: IS-4030**,** 2 x 1.0 mL each of levels 1, 2, and 3.
IDS-iSYS Cuvettes Cube: IS-CC100, box of 960 cuvettes.
IDS-iSYS System Liquid: IS-CS100, 5 L container, ready to use.
IDS-iSYS Wash Solution: IS-CW100, 10 L container, ready to use.
IDS-iSYS Triggers Set A and B: IS-CT100, 2 x 250 mL per bottle, ready to use.
IDS-iSYS Cartridge Check System: IS-6010, ready to use.
IDS-iSYS Sample Cups (500 µL): IS-SC105.

Procedure

Reagent Cartridge
The reagents provided in the cartridge are ready to use. The Analyser automatically performs the mixing of magnetic particles to maintain homogeneity. Before a new cartridge is loaded on board the Analyser, mix the magnetic particles container by brisk rotation motion. Avoid foam formation.
The barcode is read when the cartridge is loaded on the reagent tray. If the label cannot be read by the Analyser barcode reader, a manual procedure exists to enter the barcode data (see the IDS-iSYS User Manual).
Load the cartridge on the reagent tray and wait for at least 40 minutes before starting the assay.
If the cartridge is removed from the reagent tray, store the cartridge upright at 2 - 8 °C in the dark.

Calibrators
The Intact PINP calibrators are lyophilised. Reconstitute immediately before use. Add 1.0 mL of distilled or deionised water to each bottle. Replace the stopper. Leave for 10 minutes to reconstitute with occasional gentle mixing by hand. Avoid formation of foam. Pipette approximately 200 µL of calibrators and controls into sample cups and place on the machine. Discard the material in the sample cups after use. Proceed according to the instructions of the IDS-iSYS User Manual. DO NOT return material to the calibrator vial.

Analyser Calibration
The two Intact PINP calibrators are required to perform the adjustment of the master curve. The calibrators are supplied with the kit and calibrators from another lot must not be used.

Note that to perform a master curve adjustment controls MUST be run at the same time as the calibrators.

All data required for the calibration of the cartridge batch can be found on the mini CD. Use calibrator levels A and B to adjust the master curve to the reagents on board the Analyser. Check for the presence of an Intact PINP cartridge on the reagent tray and the availability of the cartridge master curve in the database. If the data for the lot of calibrators is not available on board the Analyser, load the data using the mini CD provided with the calibrator.

Start the immunoassay calibration on the IDS-iSYS Analyser according to the IDS-iSYS User Manual. The calibration is carried out in triplicate. RLU CVs of >5 % will result in a failed calibration. One replicate may be removed to meet the calibration requirements. As stated above, please note that controls must also be run. Verify and approve the calibration according to the calibration status displayed in the calibration windows and discard the calibrator from the sample tray after use.

Calibration
The calibrators of this kit are traceable to the manufacturer's working calibrators which are prepared from highly purified PINP whose value is assigned using amino acid composition analysis.

Calibration Frequency
A new calibration is required:
- Each time a new lot of cartridges is loaded on board.
- Each time a new lot of trigger or cuvettes is used.
- When the control values do not fall within the defined ranges.
- When the calibration has expired.
- After Analyser service.

Verification of the calibration is automatic and managed by the Analyser.

Quality Control
Use the IDS-iSYS Intact PINP Control Set for quality control. To ensure validity of results at least three controls with varying levels of intact PINP should be measured. Other suitable control material can be used in addition to the IDS-iSYS Intact PINP Control Set. Controls should be tested at (or near) the beginning of every run containing patient samples and also during calibrations, or according to local regulations. It is recommended that the controls be routinely run in duplicate. Laboratories should test controls at least once per shift.

Refer to the IDS-iSYS Intact PINP Control Set IFU for preparation and handling instructions.

Determination of Sample intact PINP levels
Process samples according to the IDS-iSYS User Manual.

Calculation of Results

The intact PINP concentration of each sample is calculated automatically. The display of the concentrations (screen or printed) is produced upon user request.
The IDS-iSYS Intact PINP Assay uses a 4-parameter logistic curve fit (4PL) to calculate the intact PINP concentrations.

Measurement Range (Reportable Range)

The reportable range of the assay is 2 - 230 ng/mL. Any value that reads below 2 ng/mL should be reported as "< 2 ng/mL".

Dilution

Samples with intact PINP concentrations above the reportable range should be diluted manually with a low concentration human serum sample in a ratio of 1 in 2. The results for diluted samples must be multiplied by the dilution factor 2 and corrected for the concentration of the low sample.

Limitations of Use

1. As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
2. The performance characteristics of this assay have not been established in a paediatric population.
3. The following substances do not interfere in the IDS-iSYS Intact PINP Assay when the concentrations presented in the following table are below the stated threshold.

Potentially Interfering Agent	Threshold Concentration
Lipid	2803 mg/dL
Bilirubin	200 mg/dL
Haemoglobin	500 mg/dL
Biotin	300 nmol/L

4. The hook effect was tested using concentrations of PINP up to 2350 ng/mL. No hook effect was observed.

Expected Values

Each laboratory should determine ranges for their local population.

The following range was determined using the IDS-iSYS Intact PINP Assay and is provided for information only. The 95 % reference interval for normal adults, collected from 150 apparently healthy adults, was calculated by a non-parametric method following the NCCLS guideline C28-A2, "How to Define and Determine Reference Intervals in the Clinical Laboratory".

Normal Adults 27.7 - 127.6 ng/mL (n=150)

Performance Data

Representative performance data are shown. Results obtained at individual laboratories may vary.

Sensitivity
The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 60 blanks and 120 low level samples.

LoB	0.5 ng/mL
LoD	<1.0 ng/mL
LoQ	<1.0 ng/mL

Precision
Precision was evaluated in accordance with a modified protocol based on CLSI EP-5A2, "Evaluation of Precision Performance of Quantitative Measurement Methods". Three serum controls were assayed using three lots of reagents in quadruplicate once per day for 20 days on two analysers.

Control	n	mean	Within-run		Total	
		(ng/mL)	SD	CV%	SD	CV%
1	80	14.3	0.4	2.6	0.6	4.2
2	80	44.6	1.3	3.0	2.4	5.3
3	80	100.9	3.0	3.0	4.5	4.4

Recovery
Recovery was assessed by adding intact PINP to samples prior to assay.

Sample Conc ng/mL	PINP added ng/mL	Measured ng/mL	Recovery ng/mL	Recovery %
44.3	35.3	77.1	32.8	93
44.3	74.8	115.1	70.8	95
33.5	35.3	66.4	32.9	93
33.5	74.8	101.5	68.0	91
37.3	35.3	70.8	33.5	95
37.3	74.8	105.2	67.9	91
			Mean	**93**

Linearity
Linearity was evaluated based on CLSI EP-6A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". Samples containing varying concentrations of intact PINP were assayed in duplicate. The resulting mean concentrations were compared to predicted concentrations. Samples were prepared by diluting a high patient sample with a low patient sample prior to assay.

Predicted Concentration (ng/mL)	Measured Concentration (ng/mL)	Variation	
		(ng/mL)	%
5.8	7.6	1.8	
30.5	31.5	0.9	3
55.3	55.0	-0.2	0
80.0	78.4	-1.6	-2
104.8	101.9	-2.9	-3
129.6	127.2	-2.4	-2
154.3	158.0	3.7	2
179.1	179.0	0.0	0
203.8	204.6	0.7	0

Method Comparison
The IDS-iSYS Intact PINP Assay was compared against a recognised radioimmunoassay for the quantitative determination of intact PINP, following CLSI EP-9A2, "Method Comparison and Bias Estimation Using Patient Samples". A total of 363 samples, selected to represent a wide range of intact PINP concentrations [8.7-230 ng/mL], was assayed by each method. Linear regression analysis was performed on the comparative data:
IDS-iSYS = 0.86 RIA - 1.9 (95 % CI of the slope and intercept were 0.84 to 0.88, and -3.4 to -0.3, respectively); correlation coefficient (r) = 0.98.

Specificity
The specificity was assessed with the following analytes.

Analyte	Cross-Reactivity
Intact PINP	100%
Rat / mouse PINP	<0.01%
PIINP	<0.03%
N-mid Osteocalcin	<0.02%

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